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Antibacterial Activity and β-Lactamase Stability of Temocillin

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Temocillin, a 6-α-methoxy penicillin, inhibited 90% of strains of *Escherichia coli, Klebsiella pneumoniae, Citrobacter, Proteus, Providencia, Salmonella*, and *Shigella* at a concentration of ≤16 μg/ml. *Haemophilus influenzae* and *Neisseria gonorrhoeae* were inhibited by ≤1 μg/ml. Changing the medium or pH of the cultures did not alter the minimal inhibitory concentrations, which were similar in broth and human serum, as were the minimal bactericidal concentrations. An increase in inoculum size from 10^5 to 10^7 colony-forming units increased concentration required for inhibition. Temocillin inhibited strains resistant to ampicillin, ticarcillin, cefazolin, cefamandole, and cefoxitin. Most *Pseudomonas aeruginosa* strains and other *Pseudomonas* spp. and *Acinetobacter* spp. were resistant, as were gram-positive organisms. Temocillin was not hydrolyzed by the common plasmid and chromosomal β-lactamases but inhibited them. The resistance of certain gram-negative bacilli to temocillin seemed to be a result of failure of the molecule to enter through the cell wall, since combination of temocillin with EDTA made *Pseudomonas, Acinetobacter*, and *Enterobacter* strains susceptible to low concentrations of the compound.

There has been remarkable progress in the development of new β-lactam antibiotics by the placement of side chains or groups which have altered the ability of these agents to inhibit many β-lactamase-producing isolates (8, 12). Although the semisynthetic penicillins produced in the 1960s were resistant to most of the plasmid and chromosomal β-lactamases of both gram-positive and gram-negative species, agents such as methicillin and cloxacillin failed to inhibit the *Enterobacteriaceae* since they could not penetrate the outer cell wall to reach receptor sites (17). The introduction of a 7-α-methoxy replacement in the cephalosporins, as in cefoxitin, yielded a compound that possessed high bioactivity yet was able to provide steric hinderance to β-lactamases (3). The methoxy group had not been efficiently utilized in penicillins (2) until the production of temocillin (BRL 17421), which contains a free carboxylic acid group adjacent to the side chain amide carboxyl and has a thienyl group similar to ticarcillin (Fig. 1). We evaluated the in vitro activity, β-lactamase stability, and mechanisms of bacterial resistance to this compound.

MATERIALS AND METHODS

Samples of temocillin were a gift from Beecham Laboratories. Other drugs were donated as follows: cephalothin, cefamandole, and moxalactam, Lilly Research Laboratories; cefoxitin, Merck Sharp & Dohme; cefotaxime, Hoechst-Roussel Pharmaceuticals, Inc.; cefoperazone, Pfizer, Inc.; carbenicillin and ticarcillin, Beecham Laboratories; cefazolin and ceftizoxime, Fujisawa Pharmaceuticals, Inc.

Fresh dilutions of the compounds were prepared daily in either sterile medium or distilled water. Bacterial isolates were obtained from patients hospitalized at the Columbia-Presbyterian Medical Center, New York. In some experiments the isolates tested were known to be multiply resistant to antibiotics or to contain β-lactamases or both. Some isolates had been stored frozen for a number of years.

Antimicrobial activity was measured by an agar dilution method with Mueller-Hinton agar (BBL Microbiology Systems) unless otherwise specified. A final inoculum of 10^5 colony-forming units (CFU), prepared by dilution of a fresh overnight broth culture, was applied with a replicating spot device. Broth dilutions were performed with 5×10^4 CFU in tubes of 1-ml volume. Plates or tubes were incubated at 35°C for 18 h. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic that inhibited development of visible growth on agar or in broth. The minimal bactericidal concentration (MBC) was determined by plating 0.01-ml amounts from clear broth tubes onto blood agar plates. The MBC was defined as the concentration at which there were fewer than five colonies after 24 h of incubation at 35°C. The susceptibility of streptococci was determined on Mueller-Hinton agar supplemented with 5% sheep blood. The susceptibility of *Neisseria* species and *Haemophilus* species was determined on chocolate Mueller-Hinton agar in the presence of CO_2. Tube dilutions for these species were performed with Levinthal broth. Anaerobic susceptibility was determined on Mueller-Hinton agar supplemented with sheep

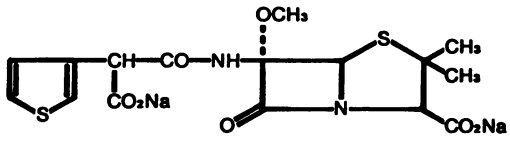

FIG. 1. Chemical structure of temocillin (BRL 17421), disodium 6β(2-carboxy-2-thien-3-ylacetamido) 6α-methoxy penicillinate.

blood and vitamin K. Anaerobic cultures were incubated for 48 h in GasPak jars (BBL).

Killing curve determinations were made in Mueller-Hinton broth with a fresh dilution of organisms from an overnight incubation. Samples were taken at selected intervals, immediately diluted in broth, and plated at several dilutions on Mueller-Hinton agar. After overnight incubations the number of CFU was determined.

Synergy studies were performed on agar with serial twofold dilutions of both agents as previously described (9). A fourfold reduction in the MIC of both agents was considered synergy (9). A fourfold reduction in the MIC of one agent and no or twofold reduction in the MIC of the other was considered partial synergy. Antagonism was defined as a fourfold increase in the MIC or a fractional inhibitory index greater than 2. Antagonism was determined by the antibiotic disk placement technique (15). Antibiotic disks containing 30 μg of temocillin or cefoxitin were placed beside disks containing cefazolin (30 μg), tobramycin (10 μg), or gentamicin (10 μg).

The β-lactamase activity of the isolates was determined by using the Glaxo chromogenic cephalosporin nitrocefin (11). The type of β-lactamase was determined by published methods (14).

β-Lactamases were characterized by isoelectric focusing techniques and were either purified or partially purified enzymes (7). Inhibition assays were performed with cephaloridine used as substrate, and the change in absorbance at 255 nm was followed spectrophotometrically (8) for the first 30 min after the enzyme was added. The reaction mixture contained 0.5 ml of 0.2 mM cephaloridine plus 0.5 ml of 0.05 M phosphate buffer (pH 7) as control and 0.5 or 0.2 mM concentrations of inhibitors. Enzymes were preincubated with inhibitors for 10 min at 30°C before the equimolar amounts of cephaloridine were added.

RESULTS

The antibacterial activity of temocillin is shown in Table 1. The concentration of temocillin which inhibited 50% of strains (MIC_{50}) for most of the *Enterobacteriaceae* was between 2 and 8 μg/ml, with the exception of *Serratia marcescens* at 32 μg/ml. The concentration which inhibited 90% of strains (MIC_{90}) was ≤16 μg/ml for *Escherichia coli, Salmonella* spp. (including *Salmonella typhi*), *Citrobacter diversus, Citrobacter freundii, Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter agglomerans, Shigella* spp., *Proteus mirabilis, Proteus vulgaris, Morganella* spp., *Providencia stuartii*, and *Providencia rettgeri*. However, some strains of *Enterobacter cloacae, Enterobacter aerogenes*, and *Serratia marcescens* were resistant, with MICs of 128 to >256 μg/ml. All of the *Neisseria gonorrhoeae* and *Haemophilus influenzae* strains, including β-lactamase-containing isolates, were inhibited by ≤1 μg/ml. Among the other species which were inhibited were *Aeromonas* spp., *Pasteurella multocida, Pseudomonas alcaligenes*, some *Serratia liquifaciens* isolates, and *Pseudomonas cepacia*.

Pseudomonas aeruginosa, Pseudomonas maltophilia, and *Acinetobacter* var *anitratus* and var *lwoffi* were generally resistant to temocillin with MICs ≥256 μg/ml. All of the gram-positive species, staphylococci, streptococci, clostridia, listeriae, and *Bacillus* spp. were resistant to the compound.

The comparative in vitro activity of temocillin and selected β-lactam drugs is shown in Table 2. Temocillin inhibited isolates of *Escherichia coli* resistant to ticarcillin and cefoperazone, but cefotaxime and moxalactam inhibited these isolates at much lower concentrations. The compound was as active as cefoxitin and cefoperazone against *Klebsiella* spp., but less active than cefotaxime and moxalactam. For all of the species tested, cefotaxime and moxalactam were more active than temocillin was. Temocillin did inhibit *Enterobacter* spp., *Providencia stuartii, Providencia rettgeri*, and *Salmonella* spp. that were resistant to ticarcillin. It inhibited strains of *Providencia, Enterobacter*, and *Citrobacter* that were resistant to cefoxitin. It also inhibited (data not shown) all of the cefamandole-resistant strains of *Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Enterobacter agglomerans*, and indole-positive *Proteus* spp., but had higher MICs against cefamandole-susceptible strains of these species.

Effect of altering test conditions. The effect of the growth medium was tested for strains of *Escherichia coli, Klebsiella, Serratia, Enterobacter*, and *Pseudomonas*. MICs were within a twofold range for Mueller-Hinton, trypticase soy, nutrient, and brain heart infusion solid media. Temocillin yielded similar MICs and MBCs for five strains each of *Klebsiella pneumoniae, Citrobacter freundii, Pseudomonas aeruginosa, Escherichia coli, Morganella morganii*, and *Providencia stuartii* at pH 6, 7, 7.4, and 8. The MIC and MBC for *Haemophilus influenzae* and *Neisseria gonorrhoeae* did not differ by more than one dilution. In the presence of 50% normal human serum the MIC and MBC values of these organisms and *Serratia marcescens* differed at most by one dilution. Incubating five species of each of these organisms under aerobic and anaerobic conditions did not change

TABLE 1. In vitro activity of temocillin

Organism (no. of isolates)	MIC (μg/ml)		
	Range	MIC_{50}	MIC_{90}
Acinetobacter spp. (32)	2–>256	>256	>256
Aeromonas spp. (5)	2–8	4	8
Bacteroides spp. (28)	8–>256	32	256
Bordetella bronchoseptica (1)	>256		
Citrobacter diversus (13)	2–32	4	8
Citrobacter freundii (17)	4–64	4	16
Enterobacter aerogenes (25)	2–>256	8	32
Enterobacter agglomerans (6)	2–>256	8	16
Enterobacter cloacae (20)	2–128	4	128
Escherichia coli (29)	2–32	4	8
Haemophilus influenzae (10)	0.1–1	0.25	0.5
Klebsiella oxytoca (12)	≤1–16	4	16
Klebsiella pneumoniae (31)	2–16	4	16
Morganella morganii (18)	2–8	2	4
Neisseria gonorrhoeae (10)	0.05–1	0.2	1
Pasturella multocida (1)	≥1		
Proteus mirabilis (23)	2–8	8	8
Proteus vulgaris (9)	2–4	2	4
Providencia rettgeri (22)	≤1–>156	2	16
Providencia stuarti (31)	≤1–>256	≤1	4
Pseudomonas aeruginosa (32)	128–>256	256	>256
Pseudomonas alcaligenes (3)	2		
Pseudomonas cepacia (3)	8–256	8	256
Pseudomonas diminuta (1)	256		
Pseudomonas fluorescens (2)	16–256		
Pseudomonas maltophilia (12)	8–>256	128	>256
Pseudomonas stutzeri (2)	32–256		
Salmonella spp. (20)	≤1–16	4	8
Shigella spp. (27)	2–32	8	16
Serratia liquifaciens (3)	<1–256	8	256
Serratia marcescens (29)	4–>256	32	>256
Bacillus subtilis (1)	>256		
Clostridium difficile (1)	>256		
Clostridium perfringens (1)	>256		
Listeria monocytogenes (5)	>256		
Staphylococcus aureus (7)	>256	>256	>256
Staphylococcus epidermidis (7)	>256	>256	>256
Streptococcus agalactiae (5)	>256		
Streptococcus bovis (1)	>256		
Streptococcus durans (1)	>256		
Streptococcus pneumoniae (5)	>256		
Streptococcus pyogenes (9)	64–>256	256	>256

the MICs determined by the agar dilution method.

The effect of inoculum size on the MIC and MBC was also determined. At 10^3 and 10^5 CFU there was a minimal increase in either the MIC or MBC (Table 3). However, at 10^7 CFU there was a marked increase in the MIC and MBC for *Enterobacter cloacae, Serratia marcescens, Pseudomonas aeruginosa, Citrobacter freundii,* and *Providencia stuartii.* At 10^5 CFU there was at most a twofold difference in the MIC or MBC.

Combination of temocillin with other agents. Since temocillin did not inhibit *Pseudomonas aeruginosa* nor gram-positive species, the effect of combining the compound with gentamicin, tobramycin, cefazolin, and ticarcillin was determined. Synergy between temocillin and the other compounds was infrequently found (Table 4). Antagonism was found for temocillin with ticarcillin in 10 of 32 isolates of *Pseudomonas aeruginosa* and with cefazolin for isolates of *Staphylococcus aureus, Pseudomonas maltophilia, Proteus vulgaris, Klebsiella pneumoniae, Enterobacter cloacae,* and *Enterobacter agglomerans.* Overall, the combination of cefazolin and temocillin showed antagonism for 22 of 56 isolates (39%). Placing antibiotic-containing disks of temocillin and cefazolin on agar plates showed a decrease of zone size similar to that achieved when disks of cefoxitin and cefazolin

TABLE 2. Comparative activity of temocillin and other antibiotics

Organism[a] (no. of isolates)	Agent	MIC (μg/ml)	
		MIC_{50}	MIC_{90}
Escherichia coli (20)	Temocillin	4	8
	Ticarcillin	>256	>256
	Cefoxitin	4	16
	Cefotaxime	0.06	0.12
	Cefoperazone	0.12	64
	Moxalactam	0.06	0.25
Klebsiella pneumoniae (30)	Temocillin	4	16
	Ticarcillin	>256	>256
	Cefoxitin	4	16
	Cefotaxime	0.06	0.12
	Cefoperazone	0.25	16
	Moxalactam	0.12	0.25
Enterobacter cloacae (20)	Temocillin	4	128
	Ticarcillin	16	>128
	Cefoxitin	>128	>128
	Cefotaxime	0.12	8
	Cefoperazone	0.25	32
	Moxalactam	0.25	4
Citrobacter freundii (17)	Temocillin	4	16
	Ticarcillin	8	128
	Cefoxitin	>128	>128
	Cefotaxime	0.12	0.5
	Cefoperazone	0.25	1
	Moxalactam	0.25	0.5
Enterobacter aerogenes (25)	Temocillin	8	32
	Ticarcillin	8	256
	Cefoxitin	>128	>128
	Cefotaxime	0.12	4
	Cefoperazone	0.25	4
	Moxalactam	0.12	2
Serratia marcescens (29)	Temocillin	32	>256
	Ticarcillin	>256	>256
	Cefoxitin	>256	>256
	Cefotaxime	1	32
	Cefoperazone	2	32

were tested against these isolates. This resistance has been reported by others for cefoxitin (12, 15). No antagonism was found for the combination of temocillin with gentamicin or tobramycin when tested against these isolates.

Killing curves. Killing curves for temocillin and cefoxitin at concentrations of twice the MIC were similar. Varying the concentration of temocillin from 4 to 32 times the MIC caused a minimal increase in the killing rate.

β-Lactamase stability and inhibition. The stability of temocillin to hydrolysis by plasmid and chromosomal β-lactamases is shown in Table 5. The most common plasmid enzymes, TEM-1 and TEM-2, did not hydrolyze the compound, nor did the staphylococcal β-lactamase. The compound was as stable as cefoxitin, slightly more stable than cefotaxime, and much more stable than cefoperazone. Only moxalactam could be considered a more stable compound.

Inhibition of the hydrolysis of cephaloridine by TEM-1, P99, and a *Morganella* sp. β-lactamase is shown in Fig. 2. Temocillin was the most effective inhibitor of TEM-1 and comparable to the other compounds in activity against the *Enterobacter* P99 and *Morganella* enzymes (4).

Effect of alteration of permeability upon activity. Since temocillin was resistant to hydrolysis by the majority of plasmid and chromosomally mediated β-lactamases whether they acted primarily as cephalosporinases or penicillinases, we determined MICs and MBCs for *Pseudomonas aeruginosa, Enterobacter cloacae, Enterobacter agglomerans*, and the *Acinetobacter* spp. in the presence of EDTA. EDTA concentrations of 1 and 10 mM significantly lowered the MIC for all of the organisms (Table 6). However, the MBCs remained elevated for all of the *Pseudomonas* and one of the *Acinetobacter* strains.

DISCUSSION

The majority of penicillins which are active against gram-negative bacteria are susceptible in various degrees to hydrolysis by various β-

TABLE 2—*Continued*

Organism[a] (no. of isolates)	Agent	MIC (μg/ml)	
		MIC_{50}	MIC_{90}
	Moxalactam	0.5	8
Providencia stuartii (31)	Temocillin	≤1	4
	Ticarcillin	256	>256
	Cefoxitin	8	32
	Cefotaxime	0.05	0.2
	Cefoperazone	4	32
	Moxalactam	0.1	0.1
Morganella morganii (18)	Temocillin	2	4
	Ticarcillin	2	32
	Cefoxitin	4	8
	Cefotaxime	0.25	0.5
	Cefoperazone	4	32
	Moxalactam	0.25	0.5
Providencia rettgeri (22)	Temocillin	2	16
	Ticarcillin	2	>256
	Cefoxitin	16	64
	Cefotaxime	0.5	4
	Cefoperazone	2	16
	Moxalactam	0.5	4
Salmonella spp. (20)	Temocillin	4	8
	Ticarcillin	>128	>128
	Cefoxitin	8	32
	Cefotaxime	0.1	0.5
	Cefoperazone	4	32
	Moxalactam	0.25	0.5
Shigella spp. (17)	Temocillin	8	16
	Ampicillin	>128	>128
	Cefoxitin	8	32
	Cefotaxime	0.1	0.5
	Cefoperazone	4	32
Proteus mirabilis (13)	Temocillin	8	8
	Ampicillin	>128	>128
	Cefazolin	8	128
	Cefoxitin	2	8
	Cefotaxime	0.1	0.5

[a] All strains were β-lactamase-containing isolates that were resistant to ampicillin.

lactamases of either chromosomal or plasmid origin. The presence of a methoxy group on the β-lactam nucleus provides stability for both cefoxitin and moxalactam against attack by plasmid β-lactamases (3, 10, 16). Temocillin is a 6β-(2-carboxy-2-thien-3-ylacetamido) 6α-methoxy

TABLE 3. Effect of inoculum size on activity of temocillin

Organism	MIC and MBC (μg/ml) at inoculum size (CFU):					
	10^7		10^5		10^3	
	MIC	MBC	MIC	MBC	MIC	MBC
Enterobacter cloacae[a]	128	>256	16	16	8	8
Serratia marcescens[a]	128	>256	32	32	8	16
Morganella morganii[a]	64	128	4	8	2	2
Klebsiella pneumoniae[b]	32	128	4	4	2	2
Pseudomonas aeruginosa[a]	256	>256	8	8	4	4
Escherichia coli[b]	6.4	6.4	3.2	6.4	3.2	3.2
Escherichia coli[a]	6.4	>256	3.2	3.2	1.6	1.6
Klebsiella pneumoniae[a]	3.2	>256	1.6	1.6	1.6	1.6
Citrobacter freundii[a]	32	64	4	4	2	4
Citrobacter diversus[a]	4	4	≤1	≤1	≤1	≤1
Providencia stuartii[a]	>256	>256	16	16	4	4

[a] β-Lactamase positive.
[b] β-Lactamase negative.

TABLE 4. Synergistic activity of temocillin combined with gentamicin or cefazolin

Organism (no. of isolates)	Agents tested	Result (no. of isolates)		
		Synergy	Partial synergy or indifference	Antagonism
Enterobacter cloacae (6)	Gentamicin	0	6	0
	Cefazolin	0	5	1
Enterobacter aerogenes (6)	Gentamicin	0	6	0
	Cefazolin	0	6	0
Enterobacter agglomerans (5)	Gentamicin	0	5	0
	Cefazolin	1	2	2
Klebsiella pneumoniae (6)	Gentamicin	0	6	0
	Cefazolin	0	4	2
Pseudomonas aeruginosa (3)	Gentamicin	0	3	0
	Cefazolin	0	0	3
Pseudomonas maltophilia (2)	Gentamicin	0	2	0
	Cefazolin	0	0	2
Morganella morganii (6)	Cefazolin	1	4	1
Proteus vulgaris (6)	Cefazolin	0	4	2
Providencia rettgeri (6)	Cefazolin	0	6	0
Staphylococcus aureus (10)	Cefazolin	0	1	9
Pseudomonas aeruginosa (32)	Tobramycin	2	30	0
	Ticarcillin	0	22	10

penicillinate. It would be anticipated that the methoxy group at position 6 would protect against hydrolysis by the plasmid β-lactamases, and the acidic function at position 9 would provide protection against hydrolysis by β-lactamases which are primarily cephalosporinases, as occurs with carbenicillin and moxalactam (10,16). Temocillin inhibited β-lactamase-containing members of the *Enterobacteriaceae*, such as *Escherichia coli*, and strains of *Klebsiella*, *Salmonella*, and *Shigella* which were resistant to penicillins such as ampicillin and ticarcillin, and *Citrobacter* strains resistant to the 7-methoxy containing cephalosporin cefoxitin.

In no instance was temocillin as active as cefotaxime or moxalactam. Its greater β-lactamase stability compared with cefoperazone enabled it to inhibit some of the organisms resistant to the latter agent, but in general cefoperazone was more active on a weight basis against the *Enterobacteriaceae*.

Temocillin was remarkably stable against attack by both plasmid and chromosomal β-lactamases and inhibited TEM-1 and P99 β-lactamases.

The fact that EDTA, which removes some of the lipopolysaccharide from the cell wall of gram-negative bacilli, lowered the MICs of resistant strains of *Pseudomonas*, *Enterobacter*, and *Acinetobacter* supports the concept that the resistance of these species to temocillin is due to failure of the compound to reach its receptor site

TABLE 5. β-Lactamase stability of temocillin compared with other agents

β-Lactamase	Organism	Stability[a]				
		Temocillin	Cefoxitin	Cefoperazone	Cefotaxime	Moxalactam
TEM-1	*Escherichia coli*	0	<1	50	<1	0
TEM-2	*Escherichia coli*	0	0	60	0	0
OXA-2	*Escherichia coli*	0	0	80	0	0
OXA-3	*Escherichia coli*	0	<1	45	<1	<1
SHV-1	*Klebsiella* sp.	0	0	13	0	0
PSE-1	*Pseudomonas* sp.	0	0	15	0	0
PSE-2	*Pseudomonas* sp.	5	10	165	20	0
PSE-3	*Pseudomonas* sp.	7	10	225	15	5
PSE-4	*Pseudomonas* sp.	1	<1	5	<1	<1
P99	*Enterobacter* sp.	0	0	10	<1	0
—	*Morganella* sp.	3	<1	0	<1	<1
—	*Bacillus cereus*	0	0	25	0	0
—	*Staphylococcus aureus*	0	0	0	0	0
Sab-Ab[b]	*Pseudomonas* sp.	0	0	0	0	0

[a] Based upon a rate of 100 for cephaloridine.
[b] Sabath-Abraham inducible β-lactamase.

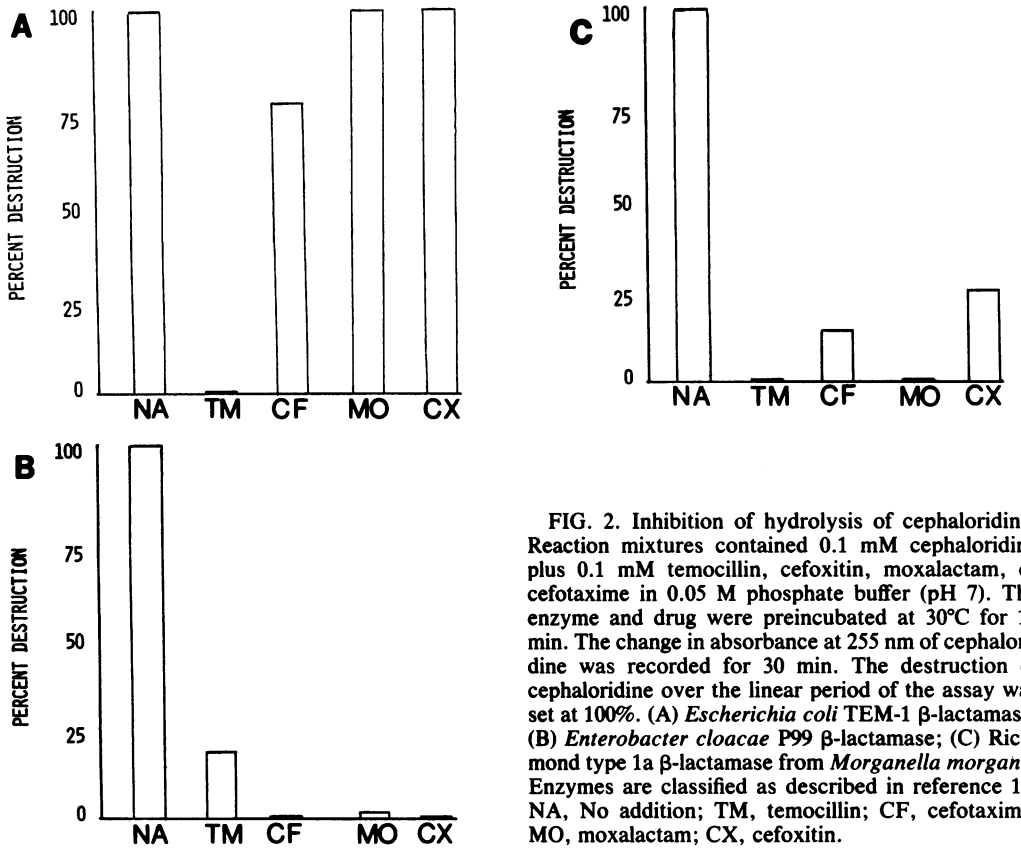

FIG. 2. Inhibition of hydrolysis of cephaloridine. Reaction mixtures contained 0.1 mM cephaloridine plus 0.1 mM temocillin, cefoxitin, moxalactam, or cefotaxime in 0.05 M phosphate buffer (pH 7). The enzyme and drug were preincubated at 30°C for 10 min. The change in absorbance at 255 nm of cephaloridine was recorded for 30 min. The destruction of cephaloridine over the linear period of the assay was set at 100%. (A) *Escherichia coli* TEM-1 β-lactamase; (B) *Enterobacter cloacae* P99 β-lactamase; (C) Richmond type 1a β-lactamase from *Morganella morganii*. Enzymes are classified as described in reference 14. NA, No addition; TM, temocillin; CF, cefotaxime; MO, moxalactam; CX, cefoxitin.

and not to destruction by β-lactamase or to failure to bind to penicillin-binding proteins.

We are in the process of investigating the precise basis for the resistance of gram-positive species to this compound, but it is probably due to its poor binding to the penicillin-binding proteins of gram-positive species, which has been seen with other compounds containing a methoxy group at position 6 or 7 on the β-lactam nucleus, or to an acidic function at position 9 or 10 of the acyl side chain (5).

The precise meaning of the antagonism of temocillin and certain other β-lactams needs further elucidation. Such antagonism has been noted with other penicillins and cephalosporins (1, 6, 12, 14).

Earlier reports (13) gave in vitro results similar to ours although with lower MICs, which may be a result of our selecting highly resistant organisms known to possess β-lactamases or to have altered permeability. The high serum levels and excellent urine concentrations that followed

TABLE 6. Effect of different concentrations of EDTA on the activity of temocillin

Organism	MIC and MBC (μg/ml) at following concn of EDTA (mM):							
	0		0.1		1		10	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Enterobacter agglomerans[a]	32	64	32	64	16	32	8	32
Acinetobacter sp.	256	256	128	256	≤0.25	32	≤0.25	32
Acinetobacter sp.	>256	>256	>256	>256	16	>256	8	256
Enterobacter cloacae	256	>256	64	128	4	64	4	8
Pseudomonas aeruginosa	>256	>256	256	>256	8	256	≤1	256
Pseudomonas aeruginosa	>256	>256	>256	>256	≤1	256	≤1	256
Pseudomonas aeruginosa	>256	>256	>256	>256	64	>256	≤1	>256
Pseudomonas aeruginosa	256	>256	256	>256	≤1	>256	≤1	>256

[a] Resistant to ticarcillin, cefotaxime, cefoperazone, ceftazidime, and moxalactam.

parenteral administration of temocillin (13) suggest that the compound would be effective in the therapy of infections caused by β-lactamase-producing *Enterobacteriaceae*. However, its ultimate role in chemotherapy awaits clinical studies.

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